(a) expressing a recombinant precursor protein in a host cell, the precursor protein comprising the target protein fused to an intein and a binding protein domain, the intein being selected from a naturally occurring intein, an intein derivative or an intein mutant, wherein the intein is capable of thiol induced cleavage;

(b) cleaving the precursor protein in the presence of a thiol reagent so as to form the target protein having a C-terminal thioester;

(c) preparing a synthetic peptide having a marker and an N-terminal cysteine; and

(d) ligating the target protein with the synthetic peptide for labelling the target protein.

A marked-up version of the claims is attached hereto.

REMARKS

Claims 7 and 12-27 are pending in the Application. Claims 12, 17, 18, 22 and 25 have been amended and claim 7 has been cancelled. Applicants believe that the amendments are supported in the Application and no new subject matter has been added.

Applicants have amended the specification to include a cross reference to U.S. Application Serial No. 09/249,543 filed February 12, 1999 which is a Continuation-In-Part of U.S. Application Serial No. 08/811,492 filed March 5, 1997 and of U.S. Provisional Application Serial No. 60/102,413 filed September 30, 1998 wherein Ming-Qun Xu is a common inventor on all three Applications.

With respect to double patenting, Applicants disclaim any additional portion of time awarded to the present claims with respect to the corresponding U.S. Application Serial No. 09/249,543 according to 37 C.F.R. §1.321.

REJECTION UNDER 35 U.S.C. §102 and 35 U.S.C. §103

The priority date of the present claimed invention as amended by cross-reference predates the references cited by the Examiner, namely Severinov and Muir (June 26, 1998), Muir et al. (April 1, 1998). The Chong reference was published less than 12 months prior to the date of the Application (after January 24, 1997) and as such is not a valid reference under 35 U.S.C. §102(b). Moreover, subject matter in Chong et al. is described in Example 23 of the U.S. Application Serial No. 08/811,492 Application (now U.S. Patent No. 5,834,247) for which Chong is a named inventor. In light of the above,

applicants request that the Examiner waive the present rejections under §102 and §103.

REJECTION UNDER 35 U.S.C. §112 FIRST AND SECOND PARAGRAPH

The Examiner has objected to the following terms:

(a) Mis-spelling of "nucleic acid" in claim 18.

Claim 18 has been amended.

(b) "Obtain" or "obtaining" as indefinite in claim 12. Applicants have noted the Examiner's proposal namely "a method of preparing an expressed protein from a recombinantly expressed precursor protein" and have amended the claims accordingly.

(c) Claim 12 lacks antecedent basis for "the expressed precursor protein" This has been corrected.

(d) "Synthetic fragment" is deemed to be indefinite in claim 22. This has been amended to recite "synthetic peptide".

(e) "Linked to a thiol inducible cleavage agent" is determined to be indefinite in claim 22. The claim has been amended to recite "intein".

(f) "Precursor protein" in claim 12: Examiner requests that the claims should include how an intein is placed in a precursor protein that does "not normally have an intein". The claim has been amended accordingly.

(g) "Preparing a plasmid" in claim 17 should describe how elements in the plasmid are operably linked to open reading frames. Claim 17 has been amended.

The Examiner has objected to the scope of the claims in a lack of adequate written description rejection. In response to these objections:

(a) Claim 7 claiming cyclic peptides has been cancelled.

(b) Claims 12, 17, 22 and 27 include the requirement that an "intein [be] selected from a naturally occurring intein, an intein derivative or an intein mutant where the intein is capable of thiol induced cleavage". This requirement is supported in the above application by, for example, disclosures on pages 6-7 and page 8.

(c) Dependent claims 13-16 are believed to be properly supported in the description and follow in proper form from the amended independent claim 12.

(d) The term "removed" has been substituted by the term "cleavage" in claim 12.

(e) Claim 17 and 18 have been amended and dependent claims 18-21 are believed to follow in proper form from claim 17 and with support in the description. For example, amended claim 17 is enabled by the description on pages 7 and 12. Amended claim 17 is directed to a precursor protein (protein precursor) which refers to the protein prior to cleavage of the intein (see for example, pages 5 and 7).

(f) Claim 17 has been amended so as to no longer include the phrase "Cleavage agent sequence". The Examiner suggested limiting cleavage agent to "intein". The claim has been amended accordingly.

(g) The method of Claim 22 has been amended where the phrase "thiol inducible cleavage agent" has been replaced and the claim now recites " inteins" and "intein mediated cleavage". Claims 22-24 do not refer to a synthetic fragment of any substance but instead specifically refers to a synthetic peptide.

(h) Claim 25 has been amended and now refers to an intein containing precursor protein. Consequently, the objections to claims 26 and 27 based on their dependency to claim 25 are now moot.

Conclusion

For the reasons set forth above, Applicants respectfully submit that this case is in condition for allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned Attorney would appreciate the opportunity to do so. Thus, the Examiner is hereby authorized to call the undersigned collect at the number shown below.

Respectfully submitted,

NEW ENGLAND BIOLABS, INC.

Date: 12/23/02

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MARKED-UP VERSION OF THE CLAIMS

12. (amended) A method for [obtaining an expressed] preparing a target protein with a C-terminal thioester, comprising:

(a) [obtaining the expressed] expressing a recombinant precursor protein in a host cell, [having an intein, an intein derivatives or mutants thereof; and] the precursor protein comprising the target protein fused to an intein and optionally a binding protein domain, the intein being selected from a naturally occurring intein, an intein derivative or an intein mutant, wherein the intein is capable of thiol induced cleavage; and

(b) [reacting] contacting the expressed precursor protein with a thiol reagent and inducing cleavage of the intein from the precursor protein so as to [(i) to remove the intein and (ii) to obtain the expressed protein with] form the target protein having the C-terminal thioester.

17. (amended) A method for expressing a recombinant precursor protein, comprising:

[inserting a protein encoding nucleic acid sequence into the plasmid upstream of an intein encoding nucleic acid

sequence, wherein the cleavage agent sequence is optionally upstream to a binding protein encoding nucleic acid sequence.]

inserting a nucleic acid sequence encoding a target protein into a plasmid at a multiple cloning site located upstream of and in frame with a fusion gene encoding an intein and a binding protein domain wherein the intein is selected from a naturally occurring intein, an intein derivative and an intein mutant modified intein; and

introducing the plasmid into a host cell for expressing the recombinant precursor protein.

18. (amended) The method of claim 17, wherein the binding protein encoded by the nucleic acid [sequence] is a chitin binding protein [encoding nucleic acid sequence].

22. (amended) A method of ligating a synthetic peptide [fragment*] in vitro* to an inactive [expressed] protein so as to restore protein activity, comprising:

(a) expressing in a host cell, [inactive truncated form of] the protein [linked to a thiol cleavage agent; and cleavage agent; and cleaving the protein in the presence of a] fused to one of an intein, an intein derivative or an intein mutant intein, wherein the intein is capable of thiol [reagent so as to form an expressed protein with a C-terminal thioester] induced cleavage;

(b) [preparing a synthetic peptide having an N-terminal cysteine; and] inducing intein mediated cleavage of the protein by adding a thiol reagent so as to form a C-terminal thioester on the protein;

(c) [ligating the inactive form of the protein with the synethetic peptide to restore protein activity] preparing a synthetic peptide having an N-terminal cysteine; and

(d) ligating the inactive form of the protein to the synthetic peptide to restore protein activity.

25. (amended) A method of labeling a [an expressed] target protein, comprising:

(a) expressing a recombinant precursor protein [linked to a thiol inducible cleavage agent and cleaving the protein in the presence of a thiol reagent so as to form an expressed protein with C-terminal thioester] in a host cell, the precursor protein comprising the target protein fused to an intein and a binding protein domain, the intein being selected from a naturally occurring intein, an intein derivative or an intein mutant, wherein the intein is capable of thiol induced cleavage;

(b) [preparing a synthetic peptide fragment having a marker and an N-terminal cysteine; and] cleaving the precursor protein in the presence of a thiol reagent